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ORIGINAL ARTICLE

Kinetics and mechanistic approach to the chromic acid oxidation of L-tryptophan with a spectral detection of chromium(III) product

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KEYWORDS

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L-tryptophan;
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Abstract The kinetics of chromic acid oxidation of L-tryptophan in H_2SO_4 medium at a constant ionic strength of 0.6 mol $dm^{-3}$and at 25 °C have been investigated spectrophotometrically. The reaction exhibits a 3:2 stoichiometry (tryptophan:chromic acid). The reaction shows a first order dependence on [chromic acid], a fractional-first order dependence on [tryptophan] and fractional-second order dependence on [acid]. Increasing ionic strength and dielectric constant had no significant effect on the oxidation rate. The final oxidation products of tryptophan were identified as the corresponding aldehyde (indole-3-acetaldehyde), ammonium ion and carbon dioxide. A spectral evidence for the formation of chromium(III) product was obtained. The experimental results suggest formation of an intermediate complex between the protonated tryptophan and chromic acid which decomposes in the rate-determining step to yield the products. The mechanism of such reaction has been proposed. The thermodynamic parameters with respect to the first step of the suggested mechanism were evaluated and discussed.

1. Introduction

Chromium(VI) is a well-established carcinogen and mutagen [1,2] and can be reduced to lower states by a wide variety of biological and chemical reductants [3]. Considerably interest has been shown in the chemistry of the intermediate oxidation states, Cr^{V} and Cr^{IV}, due to their observation during the oxidation of organic substrates by Cr^{VI} and implication in the mechanism of Cr-induced cancers [4–6].

The kinetics and mechanism of oxidation by chromium(VI) have been studied since chromic acid is one of the most versatile available oxidizing agents and reacts with diverse

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substrates [7–10]. Although, chromic acid has been extensively used for oxidation of inorganic [7,8,11–15] and organic compounds [16–24], a little attention has been focused on the oxidation of amino acids by this oxidant [17,24]. This fact may be attributed to the complexity resulting from the existence of various species of chromium(VI) in acidic medium as well as the instability of Cr^{V} and Cr^{IV} oxidation states through the reduction of chromium ion from hexavalent to trivalent state. This fact may not allow a mechanistic conclusion.

The study of amino acids is one of the most exciting fields of organic chemistry. They play a significant role in a number of metabolic reactions like biosynthesis of polypeptide, protein and nucleotides. Thus, the mechanism of analogous non-enzymatic chemical processes in the oxidation of amino acids is a potential area for intensive investigation [25] in order to understand some aspects of enzyme kinetics. Oxidation of α-amino acids is of great importance both from chemical point of view and its bearing on the mechanism of amino acids metabolism. Amino acids are very attractive natural ligands for both toxic and essential metal ions. Besides acting simply as effective chelators, in many cases they are also reducing agents; e.g. for metal ions such as Ce^{IV}, V^{V}, Co^{III} and Fe^{III}, depending on the acidity of the medium. There is also an indication that some amino acids may play an important role in the chemistry of chromium, especially in the environment, where Cr^{VI} may present a serious hazard because of its mutagenic and carcinogenic activity [1–3]. They have been oxidized by a variety of reagents under different experimental conditions and they often undergo oxidative decarboxylation and deamination [24–36].

Tryptophan (Trp) is an essential amino acid that is required in human diet. Only the L-stereoisomer of tryptophan is used in structural or enzyme proteins, but the D-stereoisomer is occasionally found in naturally produced peptides (for instance the marine venom peptide) [37]. Tryptophan functions as a biochemical precursor for the production of Serotonin (a neurotransmitter) [38,39], niacin (nicotinic acid) [40] and auxin (a phytohormone) [41].

Our literature survey reveals that the oxidation of tryptophan by different oxidants has received limited attention [33–36]. We are particularly interested to investigate the mechanism of the oxidation of such amino acid by chromium(VI) in acidic media. This mechanism is of interest because of its non-complimentary nature and the possibility of a variety of mechanisms involving reactive intermediates which have been encountered in chemical reactions. Hence, the title reaction has been investigated in order to identify the chromium(VI) intermediates and to arrive at a plausible reaction mechanism.

2. Experimental

2.1. Materials

Reagent grade chemicals and doubly distilled water were used throughout the work. A stock solution of L-tryptophan (E. Merck) was prepared afresh by dissolving an appropriate amount of the sample in bi distilled water. Required solution of the oxidant was freshly prepared before each experiment and it was standardized spectrophotometrically. All other reagents used were of analytical grade and their solutions were prepared by dissolving requisite amounts of the samples in doubly distilled water.

2.2. Kinetic measurements

All kinetic measurements were performed under pseudo-first order conditions with the tryptophan concentration being greater than the chromic acid concentration. The ionic strength, I, of the reaction mixture was adjusted to 0.6 mol dm^{-3} using Na_2SO_4. The reaction temperature (25 °C) was controlled within ±0.1 °C. The rate of disappearance of chromium(VI) was followed by monitoring the decrease in absorption spectrophotometrically at its absorption maximum, λ_{max} = 350 nm, where Cr^{VI} absorbs to a considerably greater extent than any of the other reactants and products. It was verified that there is no interference from other reagents at this wavelength. The spectrophotometer, Shimadzu UV-1800 PC automatic scanning double-beam, had a cell compartment kept at constant temperature by circulating water from a thermostat. Solutions of chromium(VI) and the mixture containing tryptophan and acid were separately thermostated for nearly 1 h. Chromium(VI) was then added to the mixture, the overall reaction mixture was transferred to the cell of path length 1 cm, and 3–4 experimental readings were taken in each run. The pseudo-first order rate constant was calculated as the plot of ln(absorbance) versus time. The gradients of such plots were calculated by the least-squares method. The rate constants were reproducible to within 4%.

3. Results

3.1. Spectral changes

The spectral changes associated with the tryptophan oxidation by chromium(VI) in acidic medium are shown in Figs. 1 and 2. The scanned spectra shown in Fig. 1 indicate gradual disappearance of Cr^{VI} – species band with time at its absorption maximum, λ = 350 nm, as a result of reduction of Cr^{VI} – species. On the other hand, there is a simultaneous growing of an absorption band located in the region *ca.* 390–470 nm with an isosbestic point centered at λ = 388 nm. A careful examination of the spectral scans (Fig. 2, where the reference cell contains Cr^{VI} and H^{+} of the same reaction mixture

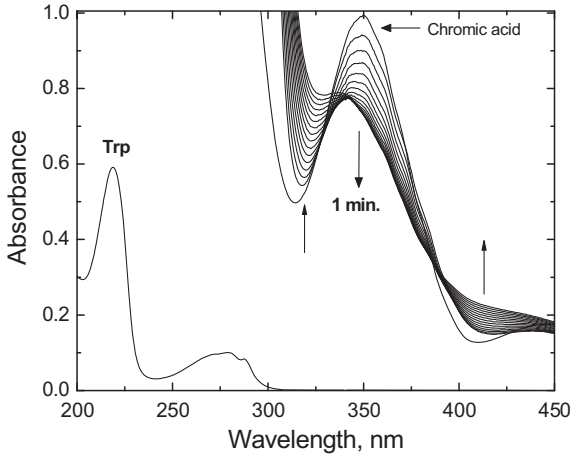


Figure 1 Spectral scans during the chromic acid oxidation of tryptophan in H_2SO_4 medium. $[Cr^{VI}] = 5 \times 10^{-4}$, $[Trp] = 12 \times 10^{-3}$, $[H^{+}] = 0.3$ and $I = 0.6$ mol dm^{-3} at 25 °C.

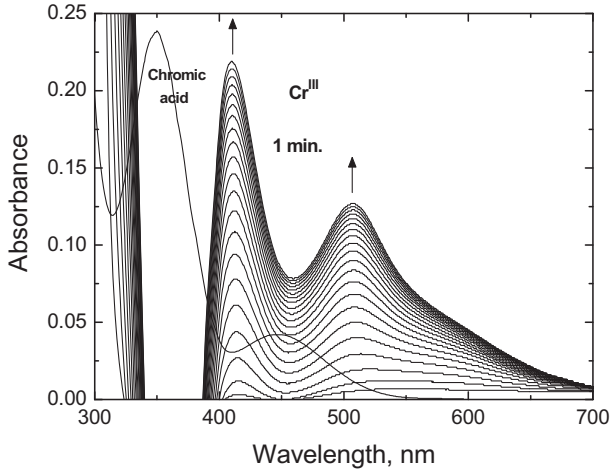
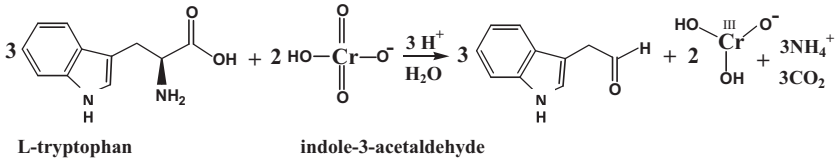


Figure 2 Spectral scans of the formation of chromium(III) product during the chromic acid oxidation of tryptophan in H_2SO_4 medium. $[Cr^{VI}] = 5 \times 10^{-4}$, $[Trp] = 12 \times 10^{-3}$, $[H^+] = 0.3$ and $I = 0.6\ mol\ dm^{-3}$ at 25 °C. Reference cell contains Cr^{VI} and H^+ of the same reaction mixture concentration.

concentration) manifests a simultaneous appearance of new bands located in the wavelength range 370–700 nm. These spectroscopic features are consistent with the formation of a chromium(III) product as reported earlier [42].

3.2. Stoichiometry and product analysis

Different sets of reaction mixtures containing varying ratios of Cr^{VI} to the amino acid were mixed at constant acidity, ionic strength and temperature and then were kept for 6 h in a closed vessel under an inert atmosphere. Estimation of the remaining Cr^{VI} concentration was performed spectrophotometrically at 350 nm. The results confirm that the stoichiometry of the overall reaction holds by the equation



L-tryptophan indole-3-acetaldehyde (1)

The stoichiometric equation is consistent with the results of product analysis. The main product, indole-3-acetaldehyde was identified by spot test [43] and was estimated quantitatively as its 2,4-dinitrophenylhydrazine derivative [44]. Other products were identified as discussed elsewhere [31,44]. Similar oxidation products with different experimental conditions have been also reported earlier [33–36]. On the other hand, the formation of Cr^{III} species was confirmed by the decrease in the oxidation rate upon addition of manganese(II) sulfate to the reaction mixture [14,20]. Such a product was obtained in most oxidation reactions of organic substrates by this oxidant [12,13].

3.3. Reaction order

The reaction orders (n) with respect to the reactants were determined from the slopes of the $\log k_{obs}$ versus log(concentration) plots by varying the concentrations (C) of substrate and acid, in turn, while keeping other conditions constant.

The concentration of the oxidant, chromium(VI), was varied in the range of 1×10^{-4} to 1×10^{-3} mol dm^{-3} at fixed [Trp], $[H^+]$ and ionic strength. Plots of ln(absorbance) versus time were linear for more than three-half lives of the reaction completion. The pseudo-first order rate constants, k_{obs}, can be evaluated from the gradients of such plots. These values were calculated by the least-squares method and were found to be independent of the initial concentration of Cr^{VI} as listed in Table 1. These results confirm that the reaction is first order in chromic acid concentration. The value of k_{obs} under the condition of $[Cr^{VI}] = 5 \times 10^{-4}$, $[Trp] = 12 \times 10^{-3}$, $[H^+] = 0.3$ and $I = 0.6\ mol\ dm^{-3}$ at 25 °C was found to be $12 \times 10^{-4}\ s^{-1}$.

The observed rate constant was determined at different initial concentrations of the reductant tryptophan keeping all other reactant concentrations constant. A plot of k_{obs} versus [Trp] was found to be linear with a positive intercept indicating that the reaction order with respect to [Trp] was less than unity. This was also confirmed by the constancy of the second-order rate constants obtained by dividing the k_{obs} values by [Trp] at fixed $[Cr^{VI}]$.

The effect of acidity on the reaction rate was studied by varying the concentration of sulfuric acid in the range 0.1–0.5 mol dm^{-3} at constant [Trp], $[Cr^{VI}]$, ionic strength and temperature. An increase in acid concentration was found to accelerate the oxidation rate (Table 1) indicating that the oxidation process is acid-catalyzed. A plot of k_{obs} versus $[H^+]^2$ was found to be linear with a positive intercept on k_{obs} axis suggesting that the reaction is fractional-second order as shown in Fig. 3. Also, a plot of log k_{obs} versus $\log[H^+]$ was found to be straight line with a slope $n = 1.85 \pm 0.04$.

3.4. Influence of ionic strength and dielectric constant

The ionic strength was varied from 0.6 to 1.0 mol dm^{-3} using sodium sulfate at constant concentrations of Trp and Cr^{VI} and at constant pH and temperature. Increasing the ionic strength had a negligible effect on the reaction rate. Similarly, at constant concentrations of reactants and at other conditions constant, t-butyl alcohol was varied from 0% to 40% (V/V) in the reaction medium. As for ionic strength, changing dielectric constant of the medium had no significant effect on the reaction rate.

Table 1 Influence of [Cr^{VI}], [Trp], [H^+] and [Mn^{II}] on the pseudo-first order rate constant value in the chromic acid oxidation of tryptophan in H_2SO_4 medium at 25 °C.

10^4 [Cr^{VI}] (mol dm^{-3})	10^3 [Trp] (mol dm^{-3})	[H^+] (mol dm^{-3})	*10^3 [Mn^{II}] (mol dm^{-3})	10^4 k_{obs} (s^{-1})
1.0	12.0	0.3	–	11.9
3.0	12.0	0.3	–	12.2
5.0	12.0	0.3	–	12.0
7.0	12.0	0.3	–	12.2
10.0	12.0	0.3	–	12.1
5.0	4.0	0.3	–	4.3
5.0	8.0	0.3	–	7.8
5.0	12.0	0.3	–	12.0
5.0	16.0	0.3	–	16.1
5.0	20.0	0.3	–	19.3
5.0	12.0	0.1	–	1.7
5.0	12.0	0.2	–	5.5
5.0	12.0	0.3	–	12.0
5.0	12.0	0.4	–	20.3
5.0	12.0	0.5	–	30.1
5.0	12.0	0.3	0.0	12.0
5.0	12.0	0.3	2.0	11.3
5.0	12.0	0.3	4.0	10.5
5.0	12.0	0.3	6.0	9.6
5.0	12.0	0.3	8.0	8.9

Experimental error ±4%.
* [Mn^{II}] = 0.0 in the first three rows (effects).

3.5. *Influence of [Mn^{II}]*

In order to check the involvement of Cr^{IV} as an intermediate during the progress of oxidation reaction, the effect of addition of manganous ions (Mn^{II}) to the reaction mixture has been examined. The reaction rate was found to decrease linearly with increasing the added Mn^{II}. The experimental results are summarized in Table 1.

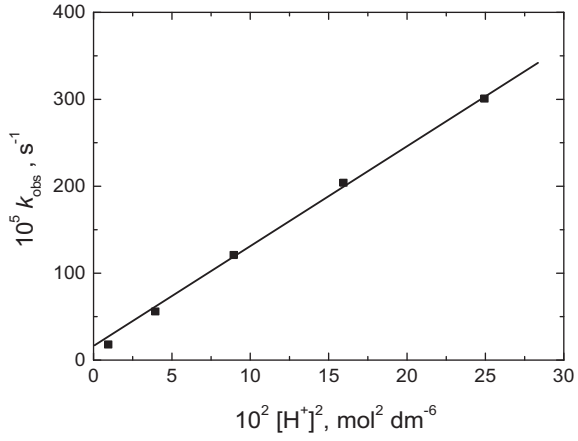


Figure 3 A plot of k_{obs} versus [H^+]^2 in the chromic acid oxidation of tryptophan in H_2SO_4 medium. [Cr^{VI}] = 5×10^{-4}, [Trp] = 12×10^{-3} and I = 0.6 mol dm^{-3} at 25 °C.

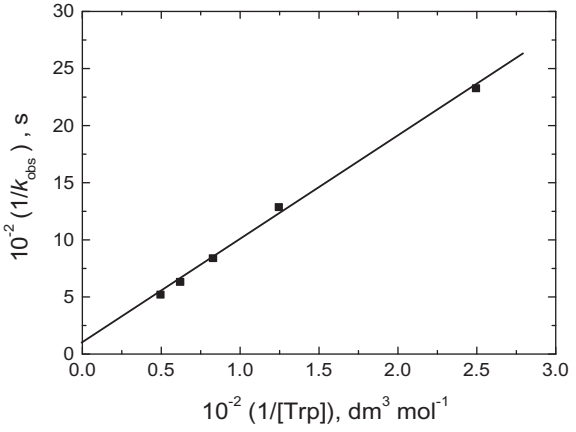


Figure 4 A plot of $1/k_{obs}$ versus 1/[Trp] in the chromic acid oxidation of tryptophan in H_2SO_4 medium. [Cr^{VI}] = 5×10^{-4}, [H^+] = 0.3 and I = 0.6 mol dm^{-3}at 25 °C.

Table 2 Values of the protonation constant K_1 along with its thermodynamic quantities in the chromic acid oxidation of tryptophan in H_2SO_4 medium.

Rate constant	Temperature (°C)				Parameter		
	20	25	30	35	$\Delta H°$ (kJ mol^{-1})	$\Delta G°_{298K}$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
K_1	1.46	1.39	1.28	1.17	−11.99	−0.816	−37.49

Experimental error ±4%.

3.6. Effect of temperature

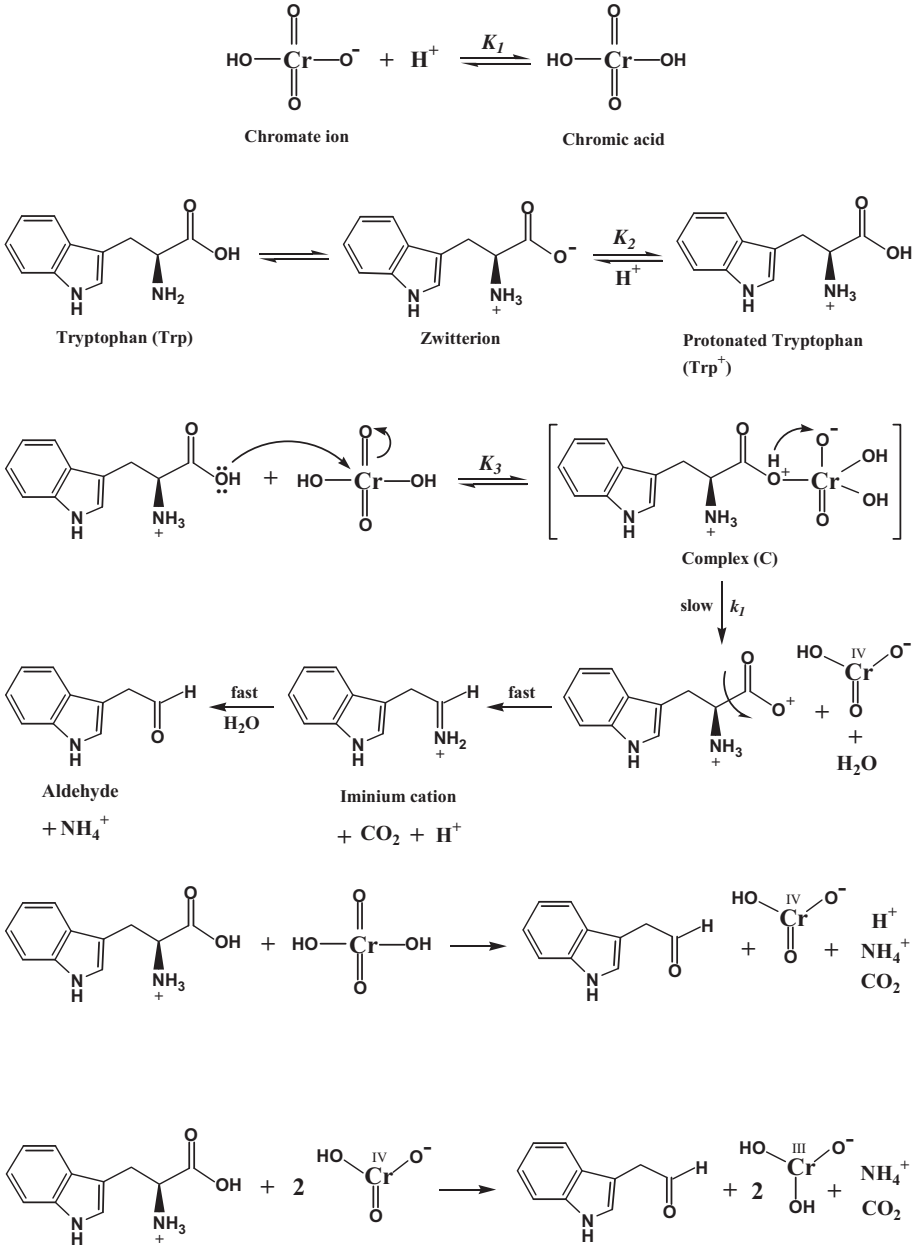
The reaction was studied at four temperatures, 20, 25, 30 and 35 °C with varying sulfuric acid concentration keeping other conditions constant. The rate was found to increase with increasing temperature. The thermodynamic parameters of the first step of the suggested mechanism were evaluated (Table 2).

3.7. Polymerization study

The possible intervention of free radicals in the reaction was examined as follows: the reaction mixtures, to which a known quantity of acrylonitrile monomer was initially added and was kept for 2 h in an inert atmosphere. On diluting the reaction mixture with methanol, no white precipitate was formed thus confirming the absence of free radical in the reaction.

4. Discussion

In redox reactions involving chromium(VI) as an oxidant, two reaction mechanisms for electron transfer may be suggested [9]. The first one corresponds to a successive one-electron transfer mechanism in a sequence. The second mechanism involves simultaneous two-electron changes in a single step. This means that both one-electron transfer and two-electron changes may be considered for oxidation of amino acids by chromium(VI) with formation of either Cr^{V} or Cr^{IV} intermediate species, respectively [13,14,20]. In the present investigation, the negative result for free radical intervention rules out the



Scheme 1 Mechanism of chromic acid oxidation of tryptophan in H_2SO_4 medium.

formation of an intermediate Cr^{V} – species. The involvement of Cr^{IV} as an intermediate during the progress of the reaction can be proved by the decrease in the oxidation rate upon addition of manganous ion to the reaction medium. This is because Mn^{II} has been recognized as a frequent tool for trapping Cr^{IV} intermediate, where the redox potential of Cr^{VI}/Cr^{III} equals 1.33 V and that of Mn^{III}/Mn^{II} equals 1.51 V [45]. This indicates that the oxidation of Mn^{II} by Cr^{VI} is unfavorable based on the thermodynamic grounds. Therefore, if Cr^{IV} intermediate is involved, the addition of Mn^{II} to the reaction mixture will remove it from the oxidation reaction and results in a decrease in the oxidation rate according to the following equation

$$Cr^{IV} + Mn^{II} = Cr^{III} + Mn^{III} \quad (2)$$

The observed decrease in the oxidation rate upon addition of Mn^{II} (Table 1) is considered as good evidence to involvement of Cr^{IV}.

Aqueous solutions of chromic acid contain ions such as CrO_4^{2-}, $HCrO_4^-$ and $Cr_2O_7^{2-}$, besides other protonated species such as H_2CrO_4, $HCr_2O_7^-$ and $H_2Cr_2O_7$ [45,46]. The ionization constant for the $HCrO_4^-$ ion, $HCrO_4^- \rightleftharpoons H^+ + CrO_4^{2-}$, is 3.0×10^{-7} mol dm^{-3}; hence, in dilute acid solution, the concentration of CrO_4^{2-} ions is negligible. Considering the values of proteolytic and hydrolytic equilibrium constants for chromium(VI) in aqueous acid medium [11,18,46,47], chromium(VI) exists mainly in the form of acid chromate, H_2CrO_4.

$$HCrO_4^- + H^+ \overset{K_1}{\rightleftharpoons} H_2CrO_4 \quad (3)$$

On the other hand, amino acids are known [48,49] to exist in zwitterions and predominantly tend to protonate in acid medium according to the following equilibria

$$Trp + H^+ \overset{K_2}{\rightleftharpoons} Trp^+ \quad (4)$$

Therefore, the fractional-second order dependence of the rate constant on the hydrogen ion concentration can be explained on the basis of proteolytic processes of both oxidant and substrate in acid medium, i.e. the protonated species of the reactants may be considered to be the more reactive species which play the main role in the reaction kinetics.

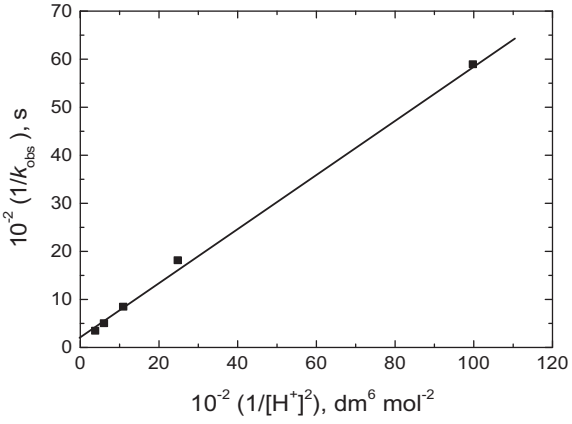


Figure 5 A plot of $1/k_{obs}$ versus $1/[H^+]^2$ in the chromic acid oxidation of tryptophan in H_2SO_4 medium. $[Cr^{VI}] = 5 \times 10^{-4}$, $[Trp] = 12 \times 10^{-3}$ and $I = 0.6$ mol dm^{-3} at 25 °C.

The present reaction between chromic acid and tryptophan in H_2SO_4 medium exhibits a stoichiometry of a 3Trp:2 Cr^{VI} with a first order dependence on $[Cr^{VI}]$ and a fractional-first order with respect to [Trp]. The fractional order dependence on tryptophan concentration can be attributed to complex formation prior to the slow step. The formation of the complex was also proved kinetically by a non-zero intercept of the plot of $1/k_{obs}$ versus $1/[Trp]$ as shown in Fig. 4. Further evidence for complex formation was obtained from the UV–Vis spectra, Fig. 1. A similar spectrophotometric observation was illustrated [22] in the reduction of chromate by glutathione in acid medium. The complex formation of chromium(VI) with tyrosine [24] and with D-xylose and L-arabinose [23] in aqueous perchloric acid medium was also reported. Hence, the most reasonable reaction mechanism which may be suggested (see Scheme 1), involves a fast complexation between the protonated substrate and chromic acid oxidant to give an intermediate complex C. This intermediate decomposes in the rate-determining step, in addition to subsequent fast steps, to give rise to the final oxidation products. The negligible effect of ionic strength and dielectric constant of the medium is consistent with a reaction that occurs between an ion and a neutral molecule [50,51], i.e. between Trp^+ and H_2CrO_4. This can be depicted by the following equations.

$$Trp^+ + H_2CrO_4 \overset{K_3}{\rightleftharpoons} [Trp - H_2CrO_4]^+ (C) \quad (5)$$

$$[Trp - H_2CrO_4]^+ \xrightarrow[\text{slow}]{k_1} \text{Products} \quad (6)$$

The suggested mechanism leads to the following rate law

$$\text{Rate} = \frac{k_1 K_1 K_2 K_3 [HCrO_4^-][Trp][H^+]^2}{\left(1 + K_1[H^+] + K_1 K_2 K_3 [Trp][H^+]^2\right)(1 + K_2[H^+])} \quad (7)$$

which explains all of the observed kinetic orders of different species. The rate law (7) can be rearranged into the following form suitable for verification

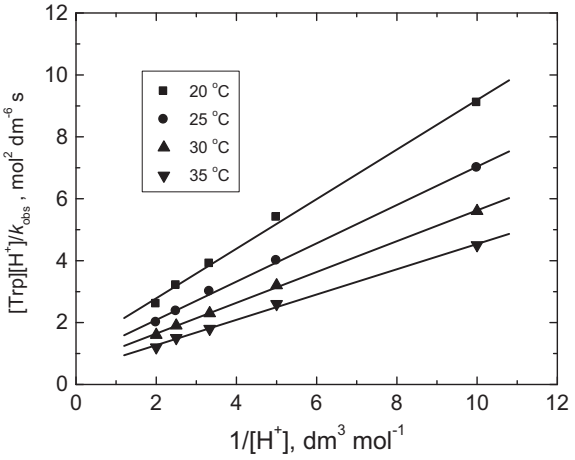


Figure 6 Plots of $[Trp]\,[H^+]/k_{obs}$ versus $1/[H^+]$ in the chromic acid oxidation of tryptophan in H_2SO_4 medium at different temperatures. $[Cr^{VI}] = 5 \times 10^{-4}$, $[Trp] = 12 \times 10^{-3}$ and $I = 0.6$ mol dm^{-3}.

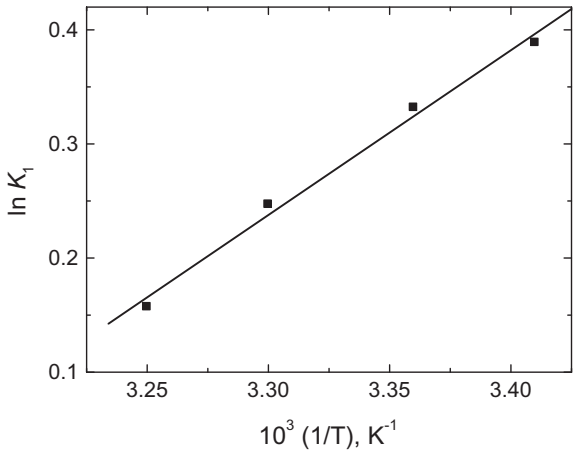


Figure 7 van't Hoff plot of K_1 in the chromic acid oxidation of tryptophan in H_2SO_4 medium. $[Cr^{VI}] = 5 \times 10^{-4}$, [Trp] = 12×10^{-3} and $I = 0.6$ mol dm^{-3}.

$$\frac{1}{k_{obs}} = \left(\frac{1 + K_1[H^+]}{k_1 K_1 K_2 K_3 [H^+]^2}\right)\frac{1}{[Trp]} + K^* \tag{8}$$

where K^* is a constant.

According to Eq. (8), a plot of $1/k_{obs}$ against 1/[Trp] at constant $[H^+]$ should be straight line with a positive intercept on $1/k_{obs}$ axis as is observed experimentally (Fig. 4). Again, a plot of $1/k_{obs}$ against $1/[H^+]^2$ at constant [Trp] also gives a straight line with a positive intercept on $1/k_{obs}$ axis (Fig. 5) confirming the validity of the proposed mechanism.

The small intercept may lead us to simplify Eq. (8) to Eq. (9)

$$\frac{[Trp][H^+]}{k_{obs}} = \frac{1}{k'}\frac{1}{[H^+]} + \frac{1}{k''} \tag{9}$$

where $k' = k_1 K_1 K_2 K_3$ and $k'' = k_1 K_2 K_3$

According to the Eq. (9), when plots, at different temperatures, were made between [Trp] $[H^+]/k_{obs}$ and $1/[H^+]$, good straight lines were observed (Fig. 6) in favor of the suggested mechanism and the rate law, from their slopes and intercepts, the protonation constant of mono hydrogen chromate ion has been evaluated by dividing k' on k'' ($K_1 = k'/k''$) and are listed in Table 2. These values were found to be in accordance with those reported previously [52].

A van't Hoff plot [53] was made for the variation of K_1 with temperature ($\ln K_1$ versus $1/T$) as shown in Fig. 7 and the values of enthalpy of reaction $\Delta H°$, entropy of reaction $\Delta S°$ and free energy of reaction $\Delta G°$ were calculated by the least squares method and are also listed in Table 2. The negative value of $\Delta S°$ indicates that there is a decrease in the randomness during the oxidation process. This leads to the formation of compacted intermediate complex and such activated complex is more ordered than the reactants due to loss of degree of freedom. Again, the negative values of both $\Delta H°$ and $\Delta G°$ indicate the exothermic formation of the intermediate and its spontaneity, respectively [54].

Unfortunately, the value of the formation constant of the intermediate complex, K_3, could not be calculated because of the non-availability of the protonation constant of tryptophan (K_2) at the same experimental conditions.

5. Conclusion

The reaction between L-tryptophan and chromium(VI) was studied in sulfuric acid medium. The oxidant, chromium(VI) exists in acid media as H_2CrO_4, which takes part in the chemical reaction. The role of hydrogen ions is crucial to the reaction. The final oxidation products of tryptophan were identified as the indole-3-acetaldehyde, ammonium ion and carbon dioxide. A spectral evidence for the formation of chromium(III) product was obtained. Mechanism proposed for the reaction is in conformity with the product, mechanistic, and kinetic studies. The thermodynamic parameters of the first step of the mechanism were evaluated and discussed.

Appendix A

According to the suggested mechanism

$$\text{Rate} = \frac{-d[HCrO_4^-]}{dt} = \frac{+d[Trp - H_2CrO_4]^+}{dt} = k_1[Trp - H_2CrO_4]^+ \tag{A1}$$

From reactions (3) – (5)

$$K_1 = \frac{[H_2CrO_4]}{[HCrO_4^-][H^+]}, [H_2CrO_4] = K_1[HCrO_4^-][H^+] \tag{A2}$$

and

$$K_2 = \frac{[Trp^+]}{[Trp][H^+]}, [Trp^+] = K_2[Trp][H^+] \tag{A3}$$

$$K_3 = \frac{[Trp - H_2CrO_4]^+}{[Trp^+][H_2CrO_4]}, \quad [Trp - H_2CrO_4]^+ = K_3[Trp^+][H_2CrO_4] \tag{A4}$$

Substituting Eqs. (A2) and (A3) into Eq. (A4) gives

$$[Trp - H_2CrO_4]^+ = K_1 K_2 K_3 [Trp][HCrO_4^-][H^+]^2 \tag{A5}$$

Substituting Eq. (A5) into Eq. (A1) leads to

$$\text{Rate} = k_1 K_1 K_2 K_3 [Trp][HCrO_4^-][H^+]^2 \tag{A6}$$

The total concentration of $HCrO_4^-$ is given by (where 'T' and 'F' stand for total and free):

$$[HCrO_4^-]_T = [HCrO_4^-]_F + [H_2CrO_4] + [Trp - H_2CrO_4]^+ \tag{A7}$$

Substituting Eqs. (A2) and (A5) into Eq. (A7) gives

$$[HCrO_4^-]_T = [HCrO_4^-]_F + K_1[HCrO_4^-]_F[H^+] + K_1 K_2 K_3 [HCrO_4^-]_F [Trp][H^+]^2 \tag{A8}$$

$$[HCrO_4^-]_T = [HCrO_4^-]_F\left(1 + K_1[H^+] + K_1 K_2 K_3 [Trp][H^+]^2\right) \tag{A9}$$

Therefore

$$[HCrO_4^-]_F = \frac{[HCrO_4^-]_T}{1 + K_1[H^+] + K_1 K_2 K_3 [Trp][H^+]^2} \tag{A10}$$

Also, the total concentration of tryptophan is given by

$$[Trp]_T = [Trp]_F + [Trp^+] + [Trp - H_2CrO_4]^+ \tag{A11}$$

Substituting Eqs. (A3) and (A5) into Eq. (A11) gives

$$[\mathrm{Trp}]_T = [\mathrm{Trp}]_F + K_2[\mathrm{Trp}]_F[H^+] + K_1K_2K_3 [\mathrm{Trp}][HCrO_4^-][H^+]^2 \quad (A12)$$

$$[\mathrm{Trp}]_T = [\mathrm{Trp}]_F(1 + K_2[H^+] + K_1K_2K_3[HCrO_4^-][H^+]^2) \quad (A13)$$

Therefore,

$$[\mathrm{Trp}]_F = \frac{[\mathrm{Trp}]_T}{1 + K_2[H^+] + K_1K_2K_3[HCrO_4^-][H^+]^2} \quad (A14)$$

In view of the high concentrations of $[H^+]$ we can write

$$[H^+]_T = [H^+]_F \quad (A15)$$

Substituting Eqs. (A10), (A14) and (A15) into Eq. (A6) (and omitting 'T' and 'F' subscripts) leads to

$$\mathrm{Rate} = \frac{k_1K_1K_2K_3[HCrO_4^-][\mathrm{Trp}][H^+]^2}{\left(1 + K_1[H^+] + K_1K_2K_3[\mathrm{Trp}][H^+]^2\right)\left(1 + K_2[H^+] + K_1K_2K_3[HCrO_4^-][H^+]^2\right)} \quad (A16)$$

In view of low concentration of $HCrO_4^-$ used, the term $K_1K_2K_3[HCrO_4^-][H^+]$ in the denominator of Eq. (A16) can be neglected. Therefore, Eq. (A16) becomes

$$\mathrm{Rate} = \frac{k_1K_1K_2K_3[HCrO_4^-][\mathrm{Trp}][H^+]^2}{\left(1 + K_1[H^+] + K_1K_2K_3[\mathrm{Trp}][H^+]^2\right)(1 + K_2[H^+])} \quad (A17)$$

Under pseudo-first order condition, the rate-law can be expressed by the equation

$$\mathrm{Rate} = \frac{-d[HCrO_4^-]}{dt} = k_{obs}[HCrO_4^-] \quad (A18)$$

Comparing Eqs. (A17) and (A18), the following relationship is obtained

$$k_{obs} = \frac{k_1K_1K_2K_3[\mathrm{Trp}][H^+]^2}{\left(1 + K_1[H^+] + K_1K_2K_3[\mathrm{Trp}][H^+]^2\right)(1 + K_2[H^+])} \quad (A19)$$

and with rearrangement we obtain

$$\frac{1}{k_{obs}} = \left(\frac{1 + K_1[H^+]}{k_1K_1K_2K_3[H^+]^2}\right)\frac{1}{[\mathrm{Trp}]} + K^* \quad (A20)$$

where K^* is a constant $\left(K^* = \frac{1+K_1[H^+]+K_1K_3[\mathrm{Trp}][H^+]+K_1K_2K_3[\mathrm{Trp}][H^+]^2}{k_1K_1K_3[\mathrm{Trp}][H^+]}\right)$

The small intercepts (in Fig. 7) may lead us to simplify Eq. (A20) to Eq. (A21)

$$\frac{[\mathrm{Trp}][H^+]}{k_{obs}} = \frac{1}{k'}\frac{1}{[H^+]} + \frac{1}{k''} \quad (A21)$$

where $k' = k_1K_1K_2K_3$ and $k'' = k_1K_2K_3$

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